The calm after the cytokine storm: lessons from the TGN1412 trial

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Commentary

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The calm after the cytokine storm: lessons from the TGN1412 trial

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In March 2006, a phase I study of the superagonistic anti-CD28 antibody TGN1412 caused a massive cytokine storm and multiorgan failure in six healthy human volunteers. Such a profound impact on the immune system was not predicted by preclinical animal studies. In a study from this issue of the JCI, Müller et al. treated rats with the superagonistic anti-CD28 antibody JJ316 and found that it rapidly induced a marked T cell lymphopenia by trapping T cells in the spleen and lymph nodes (see the related article beginning on page 1405). This dramatic redistribution of T cells simulated the profound T cell lymphopenia observed in human recipients of TGN1412. In contrast, JJ316 treatment in the rats did not reproduce the massive cytokine storm observed following TGN1412 administration to the human volunteers. These results point to similarities as well as differences between rodents and humans in the immunological effects of superagonistic anti-CD28 antibody treatment and raise further questions about how best to design preclinical studies that can better predict the risks of novel immunotherapeutics in humans.

A calm of careful retrospection has taken over the initial shock of learning about the devastating effects of a massive cytokine storm in six human volunteers who received a single infusion of the humanized monoclonal antibody TGN1412 (1). The key players in drug development are still taking stock of these events while contemplating strategies to prevent their recurrence. TGN1412, a superagonistic anti-human CD28 antibody (IgG4k), had passed the usual degree of preclinical testing, and similar reagents had shown efficacy in rodent models for treating autoimmune disease (2–4). However, TGN1412 had moved through early development on its way to a phase I trial without a deep understanding of the potential safety of this approach in humans. The superagonistic anti-CD28 antibody was able to uniquely activate T cells in the absence of a coconcurrent TCR-mediated signal, which unlocked new avenues for immunotherapy but also the possibility of unknown dangers. We may conclude, in hindsight, that these unforeseen, serious adverse events might have been anticipated in light of the high-risk nature of TGN1412’s molecular target and largely avoidable had the clinical protocol been designed with the appropriate risk-minimization strategies. In this issue of the JCI, Müller and colleagues (5) describe the effects of treatment with a superagonistic anti-CD28 antibody (JJ316) on the mechanisms of T cell redistribution and activation in the rat, providing additional insights into the immunological basis for what was observed in human subjects during the first few days after TGN1412 administration.

High stakes and lurking dangers

The story of TGN1412 ranks as a prime example of a drug development program led astray by the failure of sponsors, investigators, a clinical research organization, and regulators to ask crucial questions about the risks of an untested therapy with a novel mechanism of action. TGN1412 was in development for the treatment of B cell chronic lymphocytic leukemia, with the expectation that it would reverse the T cell deficiency in this disease, by inducing polyclonal expansion and activation of the T cell compartment. It was also hypothesized to be an expander of Tregs that might protect against the onslaught of autoimmune disease (reviewed in ref. 6). Much of the early preclinical work had been done in rats using JJ316, an antibody against rat CD28 that was functionally equivalent to TGN1412, and the same antibody used by Müller et al. in their current study (5). In rat models, treatment with JJ316 had been shown to ameliorate EAE and adjuvant arthritis through the induction of CD4+CD25+FoxP3+ Tregs with potent suppressive activity (4). These results provided a strong reason to believe that TGN1412 could become a successful first-in-class therapy with a novel mechanism of action.

However, unknown at the time was the precise mechanism by which TGN1412 activated T cells. The investigator brochure for TGN1412 surmised that the risk of developing a massive, TGN1412-induced cytokine storm was relatively low based on the results of TGN1412 testing in nonhuman primates (7). TGN1412 was known to react with CD28 from rhesus and cynomolgus monkeys, wherein it elicited a predictable, transient, and reversible expansion of CD4+ and CD8+ T cells and proliferation of CD4+CD25+ T cells (7). According to the investigator brochure, relatively modest levels of proinflammatory cytokines were produced in cynomolgus monkeys when they were treated intravenously with four weekly doses of 5 mg/kg (low dose) or 50 mg/kg (high dose) of TGN1412 (7). In these experiments, high-dose TGN1412 provoked moderate increases in the serum levels of IL-2, IL-5, and IL-6, with no significant changes in the serum levels of IL-4, IFN-γ, or TNF-α. Treatment at the lower dose resulted in only weak IL-5 and IL-6 responses. Thus, 0.1 mg/kg, or a 500th of the no-observed-adverse-effect level in nonhuman primates (50 mg/kg), was deemed to be an appropriate starting dose for the phase I trial.

The Northwick Park tragedy: an unforeseen storm

On March 13, 2006, TeGenero AG initiated its first-in-human clinical trial of TGN1412 at the Northwick Park and St. Mark’s Hospital, London, United Kingdom, under the

Nonstandard abbreviations used: ESG, Expert Scientific Group;
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As previously described by Suntharalingam et al. (1), all six of the volunteers receiving infusions in succession, 10 minutes apart. TGN1412 developed a systemic inflammation, characterized by the rapid induction of cytokines and circulating proinflammatory cytokines and ultimately lasting about 1–2 weeks, with gradual clinical improvement over the next 2–4 weeks. Regulatory authorities subsequently tested the batch of TGN1412 used in this trial and found no contamination with endotoxin or errors in manufacturing or administration, indicating that the observed cytokine storm was caused by the ligation of CD28 on human cells.

**Call to action**

What have we learned from this unfortunate mishap? Shortly after this tragedy, Kenter and Cohen raised a series of issues concerning the analysis of risk for new compounds for clinical development (8). The Medicines and Healthcare Products Regulatory Agency (MHRA) also conducted a thorough evaluation. The MHRA found no discrepancies at PAREXEL after inspecting their facilities, equipment, quality systems, and documentation. An assessment of the manufacturing process confirmed that the product met all of the batch release criteria. An Expert Scientific Group (ESG) was convened to consider what factors should be considered before first-in-human studies of agents are conducted with novel mechanisms of action. The ESG made 22 recommendations, encompassing various aspects of the drug development process (9). In their totality, these recommendations touch on the obligations of the sponsor, the appraisal of high-risk agents such as TGN1412, the qualifications and scientific expertise of the investigator, and strategies to minimize risk in first-in-human trials (see Issues raised in the ESG report related to first-in-human studies).

One aspect of the ESG report (9) dealt with the safety profile for novel agents as
Probing for answers

Since these tragic events, additional studies have been performed in an attempt to better understand the causes of the cytokine storm and the other immunological outcomes of TGN1412 treatment. Conspicuously lacking in the TGN1412 preclinical package was information about the effects of this antibody on human T cells. Warning bells would have likely sounded in the minds of the regulators if they had known that a superagonistic anti-human CD28 antibody induced rapid depletion of peripheral T cells in mice with a humanized immune system (10). We now know about these studies, as they were published after the fact. How to design experiments in vitro to predict the effects of a targeted immunotherapeutic in vivo remains a fundamental question for drug developers. To pinpoint the shortcomings in the preclinical testing, Stebbings et al. (11) set up six different protocols for presenting TGN1412 to human PBMCs or cynomolagus monkey PBMCs in vitro. They found that when TGN1412 (1.0 and 10.0 μg/well) was air dried on wells and added to human PBMCs, it induced the release of large amounts of TNF-α, IL-6, and IL-8. Also, a similar cytokine release profile was obtained when TGN1412 (0.1, 1.0, and 10 μg/well) was added in aqueous solution to a coculture of human PBMCs and endothelial cells.

Interestingly, they could not duplicate these responses using PBMCs from cynomolgus monkeys, suggesting that TGN1412 does not behave the same in nonhuman primate species as in humans. One reason this result may not be so surprising is because the extracellular domain of human CD28 differs by four amino acids from the macaque CD28 sequence, including a G68E substitution in the C'CD binding loop (i.e., the site of superagonistic anti-CD28 monoclonal antibody binding). Since TGN1412 presented to endothelial cells elicited such a powerful cytokine response, it has also been suggested that costimulatory ligands expressed on the human but not the monkey vasculature may have synergized with CD28 costimulation to induce the massive release of proinflammatory cytokines (4). Another possible explanation may derive from the recent observation that human T cells express little to none of the CD33-related siglecs, which are inhibitory signaling molecules on the cell surface that downregulate cellular activation pathways (12). In contrast, chimpanzees and monkeys express abundant levels of CD33-related siglecs on the surface of their T cells, which also show lower amounts of T cell proliferation and activation than their human counterparts upon stimulation with anti-CD3 and anti-CD28 antibodies.

CD28 superagonist–induced T lymphopenia: new insights

In this issue of the JCI, Müller et al. (5) have turned again to rats in an effort to uncover why the T cell activation, profound lymphopenia, and dramatic cytokine storm induced by TGN1412 treatment in humans was not anticipated on the basis of earlier animal experiments in the preclinical phase of TGN1412’s development. These authors found that i.v. infusion of rats with JJ316, the rat functional equivalent of TGN1412 that had been instrumental in most of the relevant preclinical studies, caused a rapid redistribution of all CD4+ T cells from the periphery to the spleen and lymph nodes (5), and therefore, simulated the T cell lymphopenia observed in humans following TGN1412 administration. The authors showed, via intravital video microscopy, that T cell motility was dramatically reduced immediately following TGN1412 administration, suggesting that the observed T cell redistribution was a consequence of T cells becoming trapped in the secondary lymphoid organs. Additional factors contributing to impaired T cell recirculation may also include the observed increase in T cell size, changes in the T cell cytoskeleton, and increased T cell adhesion to fibronectin. Müller et al. went on to show that, in addition, JJ316 impaired T cell egress from the spleen and lymph nodes via the downregulation of the sphingosine 1-phosphate receptor EDG-1 that is predominantly expressed on T cells. As such, cell-cell and cell-matrix interactions in combination with impaired T cell egress appear to play a role in the T lymphopenia, resulting from superagonistic anti-CD28 antibody treatment.

In addition to these proadhesive effects, Müller et al. found that JJ316 infusion induced two distinct waves of T cell activation (5). The first wave of T cell activation and redistribution was characterized by elevated levels of proinflammatory cytokines, including IL-17 and IFN-γ as well as increased expression of the cell surface markers CD25, CD69, and CD134. Surprisingly, JJ316 infusion elicited only mildly elevated levels of TNF-α and IFN-γ in the serum of treated rats, which produced no discernable clinical effects and were several orders of magnitude below that seen in the TGN1412-treated subjects. Therefore, JJ316 administration in rats only partially mimics the effects of TGN1412 in humans. Consequently, Müller...
Inspiring trust in drug development

Human volunteers who chose to participate in clinical trials trust in our ability to protect them as much as possible from the dangers of investigational agents. Trust is not an intangible quality but rather something real and concrete. Trust is not only about integrity but also about competence. People expect investigators, sponsors, contract research organizations, and regulatory authorities to have the talents, skills, knowledge, and capacity to carry out their responsibilities. We may lose their trust if we fail to meet our commitments to afford optimal protection of human subjects in trials. Participation in clinical trials will always have inherent risks, but hopefully lessons will be learned from the TGN1412 experience that will benefit research subjects in the future.

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9. Legrand, N., et al. 2006. Transient accumulation of human mature thymocytes and regulatory T cells with CD28 superagonist in “human immune system” Rag2−/−, CD25−/−, CD4+CD25−FoxP3− Tregs (5), involving Treg enlargement, polarization, and increased motility. The authors suggest that the cytokine storm observed in TGN1412-treated individuals was likely a consequence of the first wave of T cell activation and that the beneficial effects of superagonistic anti-CD28 antibody therapy previously observed in rodent models of autoimmune disease were likely the result of this second wave of activation that selectively affects Tregs. In summary, Müller et al. further illuminate our understanding of the mechanisms of action of the superagonist anti-CD28 antibody, and the data reinforce that this therapeutic approach will require much further investigation before it can be applied to humans.

Tetraspanin in oncogenic epithelial-mesenchymal transition

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Members of the L6 family of membrane proteins, a branch of the tetraspanin superfamily, are overexpressed in tumor cells from many types of cancers. However, direct evidence of their oncogenic activity has not been previously shown. In this issue of the JCI, Lee et al. demonstrate that overexpression of the tetraspanin superfamily member TM4SF5 in human hepatocellular carcinoma cells causes cellular phenotypic changes that resemble classical descriptions of epithelial-mesenchymal transition (EMT), with some unique aspects (see the related article beginning on page 1354). They also show that these TM4SF5-mediated effects trigger tumor formation when these cells are injected into mice. The study implicates TM4SF5, for the first time to our knowledge, in EMT oncogenic pathways of cancer progression.

Nonstandard abbreviations used: EMT, epithelial-mesenchymal transition; TM4SF5, transmembrane 4 L6 family member 5; ZO-1, zonula occludens-1.

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Over many years, cancer researchers have attempted to unlock the secrets of cancer cells by comparing the gene expression of tumor cells to that of their normal cellular counterparts. The genes so identified have in many cases proven to be important mediators of the transformed phenotype and have led in a few cases to the development of clinically useful therapeutics, such as antibodies directed against EGFR (implicated in many types of epithelial cancers) or human EGFR 2 (HER2/new; often overexpressed in breast cancer). In 1997, Gress et al. performed a large-scale screen for differentially expressed genes in tissue from individuals with pancreatic cancer compared with tissue from individuals with chronic pancreatitis and identified transmembrane 4 L6 family member 5 (TM4SF5) as a gene upregulated in pancreatic tumors (1). TM4SF5 was noted to be homologous to the integral membrane protein L6 that is also overexpressed in a variety of malignant tissues (2).